U.S. Fish & Wildlife Service

Northeast Fishery Center
Lamar, Pennsylvania

Annual Report of Biological Activities 2004

NY Governor George Pataki and Mike Glazer, aide to Congressman John Peterson-5th District of PA, assisting USFWS, NY Department of Environmental Conservation, and MD Department of Natural Resources in the experimental release of hatchery-reared Atlantic sturgeon from the Northeast Fishery Center into the Hudson River at Haverstraw Bay County Park

Staff of the Northeast Fishery Center - Lamar, PA (NEFC) came through the year 2004 with important accomplishments for the aquatic resources to which we are committed as well as making major improvements in the infrastructure of the Center. Under the leadership of Center Director, Mike Millard, activities were mostly geared towards restoration of federal trust species such as Atlantic salmon and Atlantic sturgeon. The second year of a contract agreement with the State of New York began and required that NEFC biologists perform 8 weeks of field work on the Hudson River to develop a scientifically-sound juvenile Atlantic sturgeon sampling strategy as a predictor of population trends. Other exciting sturgeon work included performing 3 experimental stockings of small numbers of NEFC-reared juveniles into the Hudson River, some of which were outfitted with sonic tags for tracking purposes. Governor George Pataki of NY personally attended one of the stocking events and had the pleasure of releasing the first sturgeon as well as assisting youngsters and others to release fish by hand. Finally, we received a shipment of 300 copies of the completed "Culture Manual for the Atlantic Sturgeon" which was 11 years in the making. Highlights of work with Atlantic salmon included the continuation of studies on the cryo-preservation of Atlantic salmon milt, calcein marking, diet improvement for wild captive salmon at North Attleboro National Fish Hatchery, genetic characterization of broodstock, and maximizing genetic diversity in progeny produced at Service salmon hatcheries for restoration stocking.

Major infrastructure improvements came in the way of installation of a new boiler which can supply heated water when needed to culture tanks and completion of an expansion of the recently-established genetics/population ecology lab which is now fully functional. NEFC also welcomes the addition of Biologist Shannon Julian, genetics lab manager, who performs much of the DNA-related laboratory work in the Population and Ecology Section as well as Jeff Kahlie, private contractor for DNA lab work. Again we are proud to report the following Biological Activities for 2004:

STUDIES PERFORMED

Study Number and Title:

LM-04-01 Evaluation of calcein-marked and unmarked Atlantic salmon fry stocked into the West Branch Sheepscot River, Maine *(finalized study of LM-01-05 and LM-02-05)*

LM-04-02 Evaluation of calcein marks on juvenile Atlantic salmon exposed to natural light held in 2 water supplies having different dissolved mineral content

LM-04-03 Growth evaluation of sub-adult Atlantic sturgeon offered one of 3 commercial diets

LM-04-04 Methanol as a potential cryo-protectant of Atlantic salmon spermatozoa

LM-04-05 The use of saline and theophylline as activator solutions during the fertilization process using cryopreserved Atlantic salmon sperm

LM-04-06 Growth and survival of Atlantic salmon *Salmo salar* fed a vegetable based diet

LM-04-07 The use oxytetracycline for validating annulus formation in pectoral spines of hatchery-reared Atlantic sturgeon *Acipenser oxyrinchus*

LM-04-08 Evaluation of Atlantic salmon kelt broodstock diets

LM-04-09 Contaminant Loads in Broodstock Fish in the Region 5 National Fish Hatchery System

LM-04-10 Evaluation of genetic diversity and relatedness for Atlantic sturgeon (*Acipenser oxyrinchus*) captively held by Maryland DNR

LM-04-11 Genetic structure of the horseshoe crab (*Limulus polyphemus*) populations in Delaware Bay

LM-04-12 Genetic characterization of an isolated population of Northern Plymouth Red-bellied Cooter (*Pseudemys rubriventris*) in Massachusetts

LM-04-13 Genetic characterization of two northern riffleshell (*Epioblasma torulosa rangiana*) populations in the Allegheny River

LM-04-14 Assessment of Watershed Scale Habitat Features on the Survival of Juvenile Atlantic Salmon

OTHER BIOLOGICAL AND RELATED INVESTIGATIONS PERFORMED:

LM04A Fish Health Inspection/Monitoring/Diagnostic Services

LM04B Sonic tagging of hatchery-reared and wild juvenile Atlantic sturgeon to track fish movements in the Hudson River

LM04C Biopsy of captive Atlantic sturgeon to determine reproductive maturity

LM04D Digital photography of calcein marks on endangered desert fish

LM04E Evaluation of calcein-marks on 5-yr-old Atlantic salmon

LM04F Sturgeon gill-netting and sampling on the Delaware River

LM04G Partnership with the Pennsylvania Fish and Boat Commission for pond culture of juvenile walleye and striped bass

LM04H Statistical/Study design consultations

LM04I Development of a non-lethal measure of estimating Atlantic salmon proximate composition for use in fish condition assessment

LM04J Northeast Fishery Center web page development

LM04K Development of water/air supply systems for freshwater mussel culture

LM04L Participation in the National Wild Fish Health Survey

LM04M Incidence and Prevalence of Infectious Salmon Anemia virus (ISAv) in Sea Run Atlantic Salmon held at Service NFHs as Broodstock

LM04N U.S. Fish and Wildlife Service Fish Health Procedures Handbook

LM04O Quality Assurance/Quality Control for Infectious Salmon Anemia virus (ISAv) Samples and Diagnostic Techniques

LM04P Fish Health Extension Services

LM04Q U.S. Fish and Wildlife Service Title 50 Revision Committee

LM04R Vaccination of pre-release Connecticut River smolts using multi-valent vaccine

LM04S Craig Brook NFH Atlantic salmon 2002 broodstock evaluation

LM04T Development of a computer program to identify genetically optimal matings for broodstock management

LM04U Craig Brook NFH Atlantic salmon 2003 broodstock evaluation

LM04V Genetic characterization and marking of Merrimack River Atlantic salmon

STUDIES / PUBLICATIONS IN WHICH THE CENTER COOPERATED:

Bay-wide tagging study to assess spawning migration and population size of horseshoe crabs in Delaware Bay. David R. Smith, U.S. Geological Survey - Biological Resources Division, Leetown Science Center, WV. Year 2 of 3. (M. Millard, USFWS Project officer)

Restoration of hatchery-impacted streams by removal of phosphorus (year 2 of 2). Phillip Sibrell, U.S. Geological Survey - Biological Resources Division, Leetown Science Center, WV. (J. Fletcher, USFWS Project officer)

Development of a non-lethal measure of estimating Atlantic salmon proximate composition for use in fish condition assessment. Conducted at NEFC. Principal Investigator: Kyle J. Hartman (West Virginia University). Co-investigators: John Sweka (NEFC), M. Keith Cox (West Virginia University).

Hartman, K.J., J. Howell, and J.A. Sweka. 2004. Diet and daily ration of bay anchovy in the Hudson River, NY. Transactions of the American Fisheries Society 113: 726-771.

Hartman, K.J., M.D. Kaller, J.W. Howell, and J.A. Sweka. *In press.* How much do valley fills influence headwater streams? Hydrobiologia 00: 1-12.

Movement, habitat use, and homing of wild pre-migrant Atlantic sturgeon in the Hudson River and of immature hatchery-reared Atlantic sturgeon of Hudson River origin released to the Hudson River. Principal investigator: Andy Kahnle (NY Dept. of Environmental Conservation). Co-investigators: Kathy Hattala (NYDEC), Michael Millard and Jerre Mohler (NEFC).

Combined use of the ASK and SHK-1 cell lines can enhance the detection of infectious salmon anemia virus*. Jill Rolland, USDA/APHIS; Deborah Bouchard, MicroTechnologies, Inc.; John Coll USFWS, (NEFC); James Winton, USGS , Western Fisheries Research Center*

STAFF PUBLICATIONS:

Bartron, M.L. & K.T. Scribner. 2004. Temporal comparisons of the genetic diversity of steelhead (*Oncorhynchus mykiss*) populations in Michigan. Environmental Biology of Fishes 69:395-407.

Bartron, M.L., D.R. Swank, E. Rutherford, & K.T. Scribner. 2004. Methodological bias in estimates of strain composition and straying of hatchery-produced steelhead in Lake Michigan tributaries. North American Journal of Fisheries Management 24(4): 1288-1299.

Jodun, Wade A. 2004. Growth and feed conversion of sub-yearling Atlantic sturgeon *Acipenser oxyrinchus* at three feeding rates. Journal of Applied Aquaculture 15 (3/4):144-150.

Sweka, J.A., M.K. Cox, and K.J. Hartman. 2004. Gastric evacuation rates of brook trout. Transactions of the American Fisheries Society 133: 204-210.

TECHNICAL INFORMATION LEAFLETS:

LM-04-01 Evaluation of calcein-marked and unmarked Atlantic salmon fry stocked into the West Branch Sheepscot River, Maine

TECHNICAL REPORTS:

Bartron, M.L. and S. Julian. 2004. Evaluation of genetic diversity and relatedness for Atlantic sturgeon (*Acipenser oxyrinchus*) captively held by Maryland DNR. Report to Maryland DNR.

Millard, M.J., J.G. Geiger, D. Kuzmeskus, W. Archambault, and T.J. Kubiak. 2004. Contaminant loads in broodstock fish in the Region 5 National Fish Hatchery System
An Informational Bulletin from the United States Fish & Wildlife Service, Northeast Region.

Sweka, J.A., J. Mohler, and M.J. Millard. 2004. Relative abundance sampling of juvenile Atlantic sturgeon of the Hudson River: Fall 2003 Progress Report to NY State Department of Environmental Conservation, Hudson River Fisheries Unit.

Sweka, J.A. Ohio River Islands National Wildlife Refuge trial night fishing evaluation. Currently under review by Regional Office.

Sweka, J.A. Ohio River Islands National Wildlife Refuge Revised Sport Fishing Plan. Currently under review by Regional Office.

Sweka, J.A. Pre-Acquisition Fishing Opportunity Plan for the Ohio River Islands NWR. Currently under review by Regional Office.

FORMAL PRESENTATIONS:

Bartron, Meredith – Genetic standardization and Atlantic salmon in Maine: management of an endangered species and aquaculture industry. Aug. 23, 2004. American Fisheries Society 134th meeting, Madison WI.

Bartron, Meredith – Genetic evaluation of alternative hatchery mating strategies. Aug. 26, 2004. American Fisheries Society 134th meeting, Madison WI.

FORMAL PRESENTATIONS (continued)

Fletcher, John – A partnership approach to aquaculture technology development. Aquaculture Advisory Committee of the Pennsylvania Department of Agriculture. Feb. 27, 2004. Harrisburg, PA

Fletcher, John – PA Aquaculture Development Center concept. Penn Aqua 2004 - Pennsylvania's Aquaculture Conference- Small Farming Alternatives, September 9-11, 2004. Harrisburg, PA

Jodun, Wade - Advanced technologies for salmon hatcheries in the Northeast: thermal performance of spiral flow heat exchangers for energy conservation. April 25-28, 2004. 60th Northeast Fish and Wildlife Conference, Ocean City, MD

Jodun, Wade - Cryopreservation of Atlantic salmon milt. April 25-28, 2004. 60th Northeast Fish and Wildlife Conference, Ocean City, MD

Millard, Michael - Policies of the U.S. Fish and Wildlife Service Fisheries Program. Nov. 11, 2004. Invited Lecture at Pennsylvania State University, School of Forest Resources.

Mohler, Jerre - Culture Manual for the Atlantic sturgeon *Acipenser oxyrinchus oxyrinchus*. Aug. 25, 2004. American Fisheries Society 134th meeting, Madison WI.

Mohler, Jerre - Status of current calcein research at the N.E. Fishery Center - Lamar, PA. August 4, 2004. Tenth Annual Drug Approval Coordination Workshop, Bozeman, MT

Sweka, John - U.S. Atlantic Salmon Assessment Committee database status and future developments. USASAC annual meeting. Feb. 24, 2004. Woods Hole, MA.

Sweka, John – US. Atlantic Salmon Assessment Committee juvenile abundance database development. Feb. 24, 2004. Woods Hole, MA.

Sweka, John – Addition of large woody debris to Appalachian streams: effects on stream habitat and brook trout populations. Aug. 23, 2004. American Fisheries Society 134th meeting, Madison WI.

Sweka, John – Contribution of terrestrial invertebrates to yearly brook trout prey consumption and growth. Aug. 25, 2004. American Fisheries Society 134th meeting, Madison WI.

Sweka, John, Jerre Mohler, and Pat Farrell - Evaluation of unique scale marking of Atlantic salmon parr with calcein (Poster Presentation). American Fisheries Society annual meeting. Aug. 23, 2004 Madison, WI.

NATIONAL COMMITTEE PARTICIPATION:

Bartron, Meredith. Atlantic Salmon Biological Review Team (BRT). (NOAA-Fisheries, USFWS, ASC, Penobscot Indian Nation) (BRT). The BRT is conducting a second status review regarding the listing of the Gulf of Maine Distinct Population Segment listing under the Endangered Species Act.

NATIONAL COMMITTEE PARTICIPATION (continued):
Coll, John – Chair of the U.S. Fish and Wildlife Service Title 50 CFR 16.13 Importation Regulations Revision Committee.

Millard, Michael - Served on the U. S. Atlantic Salmon Assessment Committee to provide information and recommendations to the U.S. NASCO delegation.

Sweka, John - Served on the U.S. Atlantic salmon Assessment Committee maintaining current assessment committee database and developing a juvenile abundance database.

OTHER SIGNIFICANT COMMITTEE PARTICIPATION:
Barbash, Patricia - Served on the Maine Fish Health Advisory Board to make recommendations to the Maine commissioners relative to fish health issues impacting wild Atlantic salmon populations and commercial aquaculture.

Barbash, Patricia.- Served on the New England Salmonid Health Committee to make recommendations to the New England Atlantic Salmon Commission (NEASC) relative to fish health issues impacting the New England states.

Bartron, Meredith-Threats Assessment Workshop for the Recovery Plan for Atlantic Salmon.

Bartron, Meredith-Craig Brook NFH Captive Broodstock Management Plan Committee.

Bartron, Meredith - Served on the Atlantic Salmon Biological Review Team

Bartron, Meredith - Served as committee chair for the Captive Broodstock Management Plan Committee (CBMPC). As part of the Atlantic salmon recovery plan, the CBMPC is drafting a formal broodstock management plan, as well as developing guidelines for maintaining captive-reared populations throughout their life cycle.

Carta, Anthony - Served as Region 5 Fisheries representative to the Northeast Region's Wage Grade Committee.

Fletcher, John (Technology Section) and John Coll (Fish Health Section) - Served as chairman and committee member, respectively, on the Design and Location Committee for the Penobscot River Atlantic Salmon Broodstock Building for Craig Brook National Fish Hatchery. The Committee met four times in FY 2004 to address: biosecurity, waste water treatment, minimization of operational costs, site selection at Craig Brook NFH, and construction budget. The work of the Committee progressed through operational review, design concept, construction site selection and review of proposed designs.

Jodun, Wade - Selected as a trainer for the Service and Maintenance Management System (SAMMS) and worked in conjunction with Gary Melvin of the National Conservation Training Center-Shepherdstown, WV to develop a training manual and set up the initial training seminar

Millard, Michael - Served on the Atlantic States Marine Fisheries Commission Horseshoe crab Technical Committee and Horseshoe Crab Stock Assessment Subcommittee.

Millard, Michael - Served as Team Leader for Chesapeake Bay/Susquehanna River Ecosystem Team

OTHER SIGNIFICANT COMMITTEE PARTICIPATION (continued):
Mohler, Jerre - Selected to serve as a member of the Atlantic States Marine Fisheries Commission Atlantic sturgeon Technical Committee.

Selmer-Larsen, Kim - Served on the Great Lakes Disease Committee to represent Region 5 relative to disease issues affecting the Great Lakes.

Sweka, John - Consulted on development of NASCO salmon habitat database, US Atlantic Salmon Assessment Committee, NASCO

Study Number: LM-04-01 (finalized study of combined LM-01-05 and LM-02-05)

Title: Comparison of mortality between calcein-marked and unmarked Atlantic salmon fry stocked in the Sheepscot River, Maine

Principal Investigator: Mike Millard, Jerre Mohler - NE Fishery Center - Lamar, Pennsylvania (NEFC)
Co-investigators:; David L. Perkins-R5; Tom King-Craig Brook National Fish Hatchery (CBNFH)

Background and Justification:

A major obstacle in evaluating the performance of fry stocked as part of Atlantic salmon restoration and recovery efforts by the U.S. Fish & Wildlife Service and it's partners has been the lack of a practical technology for marking large numbers of fry. However, recent advances have been made by NEFC in mass-marking non-feeding fry with calcein to produce an externally visible, non-lethally detectable mark visible in fin rays and other bony structures of the fish. Since this technique offers a potential solution to the problem of fry stocking assessment, studies were performed in Maine during 2001 and 2002 to field-test the technique.

Study Objectives

Using a total of 120K fry of Sheepscot River origin hatched at CBNFH, we tested the basic assumption that calcein-marked and unmarked fish had like survival and growth once released into the wild in 2001 and 2002.

Materials and Methods

Approximately 60K non-feeding fry produced at Craig Brook NFH were used in each year of the 2-year study. When fry reached a Development Index (Gaston 1988) of about 85-90 during April of each study year, roughly one-half of the study fish received a calcein mark. Each incubation tray contained fish of a lineage traceable back to individual matings of Craig Brook NFH broodstock via microsatellite markers. Offspring of 21 families grouped into 6 batches for stocking were represented in study fry. At stock-out, equivalent numbers of marked and unmarked fish of known family groups were liberated into various sites in the W. Branch of the Sheepscot. In addition, about 10K fish were also stocked in the Shorey Brook area of the Narraguagas River in 2001. After four months at large, young-of-year (YOY) were captured by electrofishing at release sites to compare catch and growth of marked vs. unmarked fish by measurement and field-examination with a battery-operated calcein detection device. During the 2001 study only, a small piece of anal fin tissue was excised from unmarked fish recovered for subsequent genetic analysis to determine if any unmarked fish were of wild origin. Numbers of calcein-marked and unmarked fish were compared using a Replicated Goodness-of-fit test (G-statistic) to test the hypothesis that marked and unmarked fry survived from stocking to capture at a 1:1 ratio. Total length data collected at each sampling station were compared between marked and unmarked fish within a site using T-tests. In the 2002 study, captured age-1 parr were also anesthetized and examined for calcein marks to determine if marks were still visible in fish at 16 months post-stocking.

Results

2001: Genotype comparisons of fin clips from unmarked fish with those of known potential parents of matings at Craig Brook resulted in hatchery parentage assignment to 110 of the 144 samples (76%). Unmarked and marked YOY did not differ significantly from the expected 1:1 ratio. Mean lengths (SD) of marked and unmarked fish were similar at all stations except at Dirigo Road where unmarked fish were larger at 68 (5) vs. 64 (5) mm for calcein-marked fish. Of the 2 sites sampled in the Narraguagas, one yielded recovery of 24 calcein-marked YOY and 28 unmarked YOY, fitting the expected 1:1 ratio. The second site yielded six marked YOY and 21 unmarked YOY, and did not fit the expected 1:1 recovery ratio. *2002:* Unmarked fish were captured at a greater than expected 1:1 ratio, but tissue samples were not taken, precluding parentage assignment. Thirteen fish from the 2001 study were found which kept the calcein mark in the wild for 16 months. Likewise, during annual parr collection in 2003, seven calcein-marked fish from the 2002 study were also captured. Analysis of length data from both study years showed that calcein marking did not adversely affect growth. Excluding labor, the cost of applying the calcein mark was about $0.01 per fish.

Study Number: LM-04-02

Title: Evaluation of calcein marks on juvenile Atlantic salmon exposed to natural light held in 2 water supplies having different dissolved mineral content

Principal Investigator: Jerre W. Mohler-NEFC
Co-investigators: John Sweka-NEFC

Background and Justification

In the quest for discovery of a long-lasting, non-lethally detectable mark which could be applied en-mass to large numbers of fish simultaneously, the Northeast Fishery Center-Lamar, PA (NEFC) began experimenting with the fluorochrome dye calcein in 1995 and performed numerous laboratory experiments which demonstrated its ability to produce a fluorescent mark in calcified structures of fish. Liberation and subsequent evaluation of calcein-marked salmon fry into the W. Branch Sheepscot River, ME in 2001 and 2002 showed that calcein-marked fish survived as well as their unmarked counterparts and that marks could be distinguished non-lethally after 4 months. In addition, sac-fry marked with calcein have retained readily-distinguishable marks as long as 3 years when maintained in indoor tanks at NEFC. Conversely, in 2003, calcein-marked fry liberated into the Woods Race tributary at NEFC exhibited poor or non-distinguishable calcein marks upon recapture only 4 months after being stocked. The difference in mark longevity between the Maine and NEFC experiments is not readily apparent but 2 different sources of calcein were used and there is some evidence that natural light can quench calcein fluorescence. Using 2 sources of calcein (Sigma and SE-MARK^{TM}) we will test the affect of natural lighting on calcein marks applied to juvenile Atlantic salmon.

Study Objectives

We used calcein from 2 sources to mark 800 Atlantic salmon young-of-year (YOY) and 400 parr in 2004 to determine whether natural light causes degradation of calcein marks over a 2-month period.

Methods

Both feeding YOY and age-1 parr were marked via immersion in 0.5% SE-MARK or SIGMA calcein solutions after a salt bath pre-treatment. Both fry and parr were maintained as follows: Culture units were 2-ft-diameter circulars with 30 fish each; 1 tank full sun (bird netting only); 1 tank shaded with top screen; 1 tank indoors. Light levels in each outdoor tank were measured with a portable light meter twice weekly. At 2 or 4-week intervals, five fish from each tank were sacrificed to obtain photomicrographs of caudal fins for analysis of calcein mark brilliance using photo analysis softwear. Statistical software was used to test the null hypotheis that mean calcein mark brilliance was the same at all light levels, calcein sources, and life stages.

Results

- Calcein mark degradation was directly proportional to the amount of exposure to natural light
- Fish marked with SE-MARK^{TM} showed consistently higher fluorescence values than those marked with SIGMA calcein.
- Even in shaded outdoor tanks, calcein marks lost a significant amount of fluorescence after 10 days.
- Fish maintained indoors retained a high level of calcein fluorescence over the 60-day study
- After 30 days, calcein marks were not visible with the hand-held detector, but some could be seen using fluorescence microscopy at 100X

Study Number: LM-04-03

Title: Growth evaluation of sub-adult Atlantic sturgeon offered one of 3 commercial diets

Principal Investigator: Jerre W. Mohler - NEFC
Co-investigators: Wade Jodun; John Sweka; Patrick Farrell – NEFC

Background and Justification

There are many commercial salmonid diets from which fish culturists can choose, but diets formulated specifically for sturgeon are limited to just a few brands. Therefore as new sturgeon diets are developed, they should be evaluated. One such diet being considered for commercial offering is marketed by Vitan, a Russian-based company which has a N. American sales division. The Vitan diet is scheduled for manufacture in the U.S. by Melick Feeds of Catawissa, PA and is available for evaluation. In addition, Corey Aquafeeds also produces a sturgeon diet (Aqua Clear) which has not been evaluated for Atlantic sturgeon. Currently, NEFC has an adequate number of domestic Hudson River sub-adult sturgeon available to use in a comparative diet study. These fish have been fed Zeigler sturgeon diet for a number of years. Should these domestic stocks become useful as broodfish for future restoration stocking efforts, it is imperative that the nutrition be maintained at levels conducive to production of good quality eggs and milt.

Study Objectives

We compared the growth response of about 75 F1-generation domestic Atlantic sturgeon from the 1998 year class offered one of three diets: Vitan, Corey, and Zeigler sturgeon diet during the 2004 growth season at NEFC.

Methods

One of 3 diets (Zeigler sturgeon, Corey Aqua Clear, or Vitan sturgeon) was offered to study fish from May to October, 2004. Three 3.7-m-diameter tanks supplied with flow-through water at the Intensive Culture building were used in the study with fish in each tank offered one of the 3 study diets. Each tank was balanced for biomass within ±5% and contained 25 individuals of the 1998 year class. Weight and length changes of individual fish were tracked throughout the study via their unique PIT tag number, therefore a repeated measures analysis was used to determine whether there were statistical differences in the effects of diet treatment on growth of study fish. Study diets consisted of pellets ranging in size from 3.8 to 6mm hand-fed twice daily and offered at a daily rate of 0.005 of the tank biomass. Feed amount offered was adjusted bi-monthly according tank biomass changes. Fish fed diets with significantly higher moisture contents may be at a disadvantage since they will receive less dry nutrient material than their study counterparts which are offered diets with low moisture. Therefore, mean percent moisture content of study diets was determined by drying 3 equal-wet-weight samples of each diet in a drying oven for 2 hours at 135±2° F. Diet moisture content was determined as a mean of at least 3 sampling periods corresponding to tank biomass inventories. Data were used to compare the feed conversion factors calculated on both “as fed” and dry feed basis.

Results

-During what should have been a high growth period, fish biomass stayed nearly the same or decreased in 2 of the 4 inventory periods for all diet treatments.

-Near the end of the study, we discovered that tank flow characteristics caused feed to accumulate near the center drain or feed drained to waste too quickly, leaving it unavailable for consumption by the sturgeon. Slower-sinking diets drained to waste more quickly than faster sinking diets

-Since residence time was not similar for test diets, effect of diet upon fish growth was not compared statistically

Study Number: LM-04-04

Title: Methanol as a potential cryoprotectant of Atlantic salmon spermatozoa

Principal Investigator(s): Wade Jodun, Pat Farrell and Kim King **-** NEFC
Co-Investigator(s): William Wayman and Greg Looney USFWS, Warm Springs Technology Center

Background and Justification

Atlantic Salmon (ATS) Restoration relies heavily upon fish culture facilities to produce fry, parr, and smolts for stocking. Despite decades of stocking, sea-run returns of ATS remain at low levels. The U. S. Fish & Wildlife Service has taken strong interest in the potential of cryopreservation techniques to maximize the conservation of available genetic material. Cryopreservation has been identified as a viable means to establish germ plasm repositories for fish production facilities when a shortage of males exist and to provide sperm from males with a known disease history for selective breeding programs. Early attempts to cryopreserve sperm in straws were unsuccessful but high fertility success (80%) has been reported with a pellet technique in which ampolues were frozen. The non-repeatable results indicate no satisfactory technique has yet been developed for cryopreserving ATS semen. The suitability of extenders supplemented with 10% methanol has been demonstrated to cryopreserve the semen of rainbow trout, lake trout and Arctic char. However, extenders supplemented with methanol have yet to be tested on ATS.

Objectives

Our objectives were to test the effect of Cloud extender with or without egg yolk supplementation and containing either methanol or DMSO on the fertilization success of cryopreserved ATS semen.

Materials and Methods

Source of Milt and Eggs.-- 5 ml aliquots of milt were collected from 20 Connecticut River F1 ATS held at White River NFH in Bethel, VT (WRNFH). Only samples with motility of at least 90% were used. Eggs were pooled from five ripe females and stored in a covered bowl at 8.0°C prior to fertilization. *Freezing of semen*.--The effect of 4 extenders on the success of cryopreservation of ATS sperm was tested. Extenders were: (1) Cloud (54.04 g/L glucose and 1.7 g/L KCl) with 5% dimethyl sulfoxide (DMSO), (2) Cloud with 5% DMSO supplemented with 13.3% egg yolk, (3) Cloud with 10% methanol (4) Cloud with 10% methanol supplemented with 13.3% egg yolk. Semen was mixed with extenders at a dilution ratio of 1:4. Diluted semen was drawn into 0.5 ml straws and placed directly into Dewars containing liquid nitrogen for storage. *Thawing of semen and fertilization*.-- On the day of spawning, individual straws were thawed at 40°C in a water bath for 7 seconds and immediately used to fertilize about 300 eggs. After fertilization, eggs were water-hardened in 50 ppm iodophore for 30 min and then placed in randomly assigned Heath tray compartments. *Incubation*. --Fresh water was introduced at the rate of 15 L/min. All eggs received a 15-min flow-through treatment of 1500 mg/L Paracide F every other day beginning on the second day of incubation and continuing until eggs were eyed. After eggs developed to the eyed stage, they were physically shocked, and survival was determined by percent eye-up.

Results

- Fertilization rates, expressed as the percentage of eyed embryos, ranged from 52.7 to 83.5 %.
- Sperm cryopreserved using Cloud with 10% methanol supplemented with 13.3% egg yolk yielded significantly higher fertilization rates (83.0%) than did sperm cryopreserved with the other three extenders.
- Fertilization rates compare favorably to those observed for eggs from the same year class fertilized with fresh milt (81.4%) and reared at WRNFH. The presence of egg yolk in extenders incorporating 10% methanol provided additional protection to salmonid sperm during the freezing and thawing processes and resulted in and increase in survival from 72.9 to 83.5%.
- Our results indicate cryopreservation has potential to be used as a tool for ATS management.

Study Number: LM-04-05

Title: The use of saline and theophylline as activator solutions during the fertilization process using cryopreserved Atlantic salmon sperm

Principal Investigator(s): Wade Jodun, Pat Farrell and Kim King **-** NEFC
Co-Investigator(s): William Wayman and Greg Looney USFWS, Warm Springs Technology Center

Background and Justification

In recent years, the USFWS has taken strong interest in using cryopreservation techniques to maximize conservation of genetic material since sea-run returns of Atlantic salmon (ATS) remain low despite decades of stocking. Several methods have been tested but no satisfactory technique has been developed for ATS sperm. Use of fertilization activators is becoming more common in salmonid breeding. These activators are buffered saline-based solutions that reportedly enhance the duration of sperm motility and have led to greater fertilization rates for several species. The duration of sperm motility has been reported higher in saline solutions than in fresh water. Some have noted the addition of methylxanthines to buffered saline activator solutions enhanced sperm motility. Similar results were seen with theophylline, a less costly methylxanthine. The fertilizing capacity of cryopreserved rainbow trout semen was increased 4-fold when fertilized in a buffered saline activator with 5 mM theophylline .

Objectives

The objective was to test the effect of various post-thaw activators on fertilization rate when used during the fertilization process in conjunction with cryopreserved ATS sperm.

Materials and Methods

Source of Milt and Eggs.-- 5 ml aliquots of milt were collected from 15 ripe Connecticut River F1 ATS held at White River National Fish Hatchery, Bethel, Vermont (WRNFH). Only samples with motility of at least 90% were used. Eggs were collected from five ripe females. Eggs were pooled and stored in a loosely covered bowl in a water bath at 8.0°C prior to fertilization. *Freezing of sperm*. - - Extenders used included: (1) Cloud extender (54.04 g/L glucose and 1.7 g/L KCl) with 5% dimethyl sulfoxide (DMSO), (2) Cloud extender with 5% DMSO supplemented with 13.3% egg yolk, (3) Cloud extender with 10% methanol (4) Cloud extender with 10% methanol supplemented with 13.3% egg yolk. Semen was mixed with extenders at a dilution ratio of 1:4. Diluted semen was immediately drawn into 0.5 ml French straws and placed directly into Dewars containing liquid nitrogen for storage. *Thawing of semen and fertilization*.-- Frozen semen was thawed at 40□C in a water bath for 7 seconds and immediately placed on eggs. A single 0.5 ml straw of extended semen was used to fertilize approximately 250 eggs. Three fertilization activator solutions were tested for each extender. Activator solutions included: (1) ovarian fluid; (2) buffered saline solution (0.9% NaCl, 0.01 M tris, and 0.02 M glycine at pH 9.0); (3) buffered saline solution with 5 mM theophylline added. After fertilization, eggs were water-hardened in 50 ppm iodophore for 30 minutes and placed in randomly assigned Heath tray compartments. *Incubation*. -- Fresh water was introduced at the rate of 15 L/min. All eggs received a 15-min flow-through treatment of 1500 mg/L Paracide F every other day beginning on the second day of incubation and continuing until eggs were eyed. After eye-up, eggs were physically shocked, and survival was determined by percent eye-up.

Results

- In the Cloud with 5% DMSO lots, eggs had greater fertilization rates using ovarian fluid (76.0%) and buffered saline solution (61.2%) as post-thaw activators as opposed to buffered saline with 5 mM theophylline (24.9%).

- Fertilization rates for the trials conducted using the Cloud with 5% DMSO supplemented with 13.3% egg yolk extender and utilizing either ovarian fluid, buffered saline solution, or buffered saline solution with 5 mM theophylline as activator solutions were 53.3% ± 5.5, 32.3% ± 9.9 and 33.14% ± 7.1 respectively.

- Highest fertilization rates were obtained using ovarian fluid as a post-thaw activator solution.

Study Number: LM-04-06

Title: Growth and survival of Atlantic salmon *Salmo salar* fed a vegetable based diet

Principal Investigator(s): Wade Jodun, Pat Farrell **-** NEFC

Background and Justification

Between 1987-1999, salmon consumption increased annually at a rate of 14% in the European Union and 23% in the U.S. Currently, over half the salmon sold globally is farm-raised. The annual global production of farmed salmon (mostly Atlantic salmon) has risen from 24,000 to more than a million metric tons in the past 20 years. This growth in production is expected to continue as demand for fish increases. However, some studies have suggested that consumption of farmed salmon may have health risks due to the possibility of bioaccumulated contaminants in the fish tissue. Other studies evaluated more than 2 metric tons of farmed and wild salmon from Europe and N. America and found significantly higher concentrations of each of the 14 organochlorine contaminants in farmed fish. Total polychlorinated biphenyls (PCBs) and dioxins were also consistently and significantly more concentrated in farmed salmon. The differences in contaminant concentrations observed between farmed and wild salmon were thought to be a function of their diet. Farmed salmon are typically fed feed high in fish oils and fish meal obtained primarily from small pelagic schooling fishes which are known to accumulate toxins such as organochlorines. In part of previous investigation, analysis of 13 commercial salmon feeds showed levels of organochlorine contaminants similar to the levels in farmed salmon. Research concluded that consumption of farmed salmon may result in exposure to a variety of bioaccumulative contaminants which pose a risk to human health. Such findings have led fish culturists to look for means to reduce, or eliminate entirely, the use of fish meal in aquaculture.

Objectives

To compare the performance of Atlantic salmon fry fed a diet composed entirely of soy, gluten and plant protein versus a traditional, widely used diet comprised of fish and animal meal.

Materials and Methods

Approximately 2,250 F1-generation ATS sac-fry of Connecticut River origin produced at NEFC were employed for this study. Fish were randomly distributed at a density of 250 fish / tank in each of 9 circular tanks (48 cm in diameter at the base and 38 cm tall). Tanks were equipped with screened internal standpipes and provided flow through water at 1.5 liters per minute. Water levels were maintained at a constant depth of 28 cm resulting in a volume of approximately 64 L. Prior to the onset of feeding, triplicate tanks were randomly assigned one of the following diet regimens: (1) Corey Starter (CS) - a commercially available, fish meal-based dry diet provided to first-feeding fry by a number of federal salmon hatcheries in the northeast, (2) Freedom Feeds starter diet (FF) - a fish feed developed and manufactured by Freedom Feeds, Urbana, Ohio whose protein source is grain, grain products, plants and plant protein products or, (3) CS offered for 2-3 weeks followed by a 2 week weaning to FF. Feed was offered at the rate of 4.5% body weight/d. Mortalities were removed and recorded prior to the initial feeding each AM. Tanks were cleaned and flushed daily. Following the initial inventory, total tank biomass was measured every 14 days to assess growth and to adjust feed ration to compensate for weight gain. Following growth inventories, each tank received a prophylactic 0.5 % salt treatment. Fish were not fed on days on which they were inventoried. Rearing continued for 40 weeks.

Results

- After 20 weeks, neither mean weight nor survival were significantly affected by diet regimen.
- Mean weight (± SD) for each of the regimens was 1.0 ± 0.1 g/fish (CS), 0.98 ± 0.1 g/fish (CF) and 0.90 ± 0.1 g/fish (FF) and
- After 20 weeks, mean survival was 71% (CS), 69% (CF) and 61% (FF).
- After 40 weeks, fish fed a constant fish meal-based diet (CS) were larger (6.3 ± 0.2 g/fish) than fish fed either the CF (4.3 ± 0.1 g/fish) or FF (3.9 ± 0.04 g/fish) diets, both of which had plant protein-based diets in the feeding regimen.
- After 40 weeks, fish offered CS had greater survival (70%) than those offered either CF (47%) or FF (41%).

Study Number: LM-04-07

Title: The use oxytetracycline for validating annulus formation in pectoral spines of hatchery-reared Atlantic sturgeon *Acipenser oxyrinchus*

Principal Investigator: Wade A. Jodun - NEFC
Co-investigators: Thomas Kehler - Penn State University; Pat Farrell - NEFC

Background and Justification

Aging of fish is routinely estimated through examination of calcified structures for growth patterns. Although a variety of skeletal parts with visible calcium depositions have been used to age sturgeon, age estimates for this group are largely based upon counts of apparent annuli visible in thin transverse sections of the pectoral fin rays. However, despite its widespread use in aging sturgeon, pectoral-fin spine technique has only recently been validated for white sturgeon *Acipenser transmontanus* and for lake sturgeon *Acipenser fulvescens.* The Atlantic States Marine Fisheries Commission identified verification of current methodology for estimating ages of Atlantic sturgeon (ASN) using fin rays as a high priority research need. Other researchers provided a partial validation of the periodicity of ring deposition in ASN when they used a solution of 25 mg oxytetracycline (OTC)/ kg of fish injected into the dorsal musculature to produce distinct marks on a small (n=5) number of known-age (4 year old), laboratory-reared ASN and observed a distinct OTC mark followed by an opaque zone three months after injection with retention at least 15 months after injection. However, for a complete validation of the technique it is first essential to track fish for at least a year to identify an annulus. Validating fish aging techniques is extremely important in fisheries biology. Without accurate aging techniques, growth rates, age at maturity, longevity predictions and estimates of age-specific data such as catch rates, fecundity, mortality, and growth rates, may be inaccurate. This information will provide further confirmation of the pectoral-spine aging technique for fish of a range of ages as well as providing a key for use with the pectoral spine aging methodology for the species.

Study Objectives

Using 50 ASN from 5-yr classes we will validate, through the application of an OTC mark, whether or not the zone preliminarily identified as an annulus (one opaque and one translucent ring) on ASN pectoral-fin spines is, in fact, an annual increment over the 2003-2004 time period, at least.

Materials and Methods

Ten (10) sturgeon from five year classes (FY 98, 96, 95, 94, 93) currently being cultured at the NEFC were injected using OTC to produce a mark of known date in skeletal structures. All fish were anesthetized with 200 ppm MS-222, measured for fork length to the nearest millimeter, weighed to the nearest gram and administered a solution of 50 mg OTC/ kg of fish injected into the dorsal musculature to produce distinct marks. Twelve months after injection, a small segment of the right pectoral was removed with a jeweler's saw. Fin spine segments were dried and a minimum of three transverse sections (thickness = 0.2 mm) were taken from each fin spine, mounted on glass microscope slides, polished, viewed with epiflourescent micropscopy and photographed to verify the existence of OTC marks and identify annual banding patterns within and among the various age groups.

Results

-Twelve months after the initial injection, all age classes of OTC-injected fish exhibited a distinct OTC mark followed by an opaque and translucent zone. Survival in all lots, including controls was $\geq$ 90%.

-The leading edge of all fins from which spines were removed had completely healed with no evidence of any long-term detrimental consequences caused by the removal process.

-These results provide further confirmation that one pair of opaque and translucent rings does, in fact, constitute an annual growth increment in Atlantic sturgeon.

- The study will extend into a 2nd year with repeated application of OTC and evaluation of the left pectoral spine section.

Study Number: LM-04-08

Title: Evaluation of Atlantic salmon kelt broodstock diets

Principal Investigator: John Fletcher, Northeast Fishery Center (NEFC)
Co-Investigators/Cooperators: Dale Honeyfield, Northern Appalachian Research Laboratory; George Ketola, Tunison Laboratory of Aquatic Science; Ann Gannam, Abernathy Fish Technology Center; Larry Lofton and Fred Yost, North Attleboro NFH; John Sweka, NEFC

Background and Justification

Reproduction from Atlantic salmon (ATS) kelts represents valuable genetic and numeric contributions to restoration fry stocking. However, survival, maturation and gamete quality of kelts has been inconsistent. The present study examines the nutritional effect of two diets (standard *vs* USGS) upon kelt reproductive success and growth. Nutritional variability and seasonal availability of raw ingredients found in the standard formulation are viewed as potential problems. Biochemistry of the standard diet was examined by comparing mineral and lipid profiles of eggs collected in 2000 from wild sea-run ATS and hatchery rejuvenated kelts on the standard diet. The analyses showed that kelt eggs were deficient in copper and selenium and contained excessive amounts of manganese relative to sea-run eggs. The standard diet, however, was found to contain high levels of copper, zinc, manganese and selenium. A five fold reduction of mineral premix was recommended to correct the mineral imbalance found in the standard diet at study inception in 2001. An alternative USGS diet formulation based upon advances in nutritional research moved to processed meals and lower levels of minerals in readily absorbed chelated form. The new protein sources are advantageous in respect to quality and availability.

Objectives

Increase the genetic contribution of the ATS kelts to Atlantic salmon recovery program by improving quality of kelt reproductive products (egg and sperm). The hypothesis of this study is that nutritional benefits derived from re-formulation of the kelt diet will result in improvement in reproductive success.

Materials and Methods

Merrimack and Connecticut River ATS kelts at North Attleboro NFH received standard and USGS diets from rejuvenation in 2001, 2002 to spawning in November, 2003. Evaluation of gamete quality as measured by survival to eye-up was determined for each mature female from a diet-river group by fertilization with milt from all males representing that group; viability of each male from a diet-river group was measured against a pooled composite of cohort eggs.

Results

Although individual performance varied, no difference ($P > 0.05$) in percent mean (SE) eye-up was found between the standard diet at 72 (3) and USGS diet at 76 (3). Likewise differences were not detected for spermatocrit or sperm motility levels nor were correlations found for these measures with viability. Data analyses relative to broodstock growth, survival, maturation, fecundity, egg size; and lipid and mineral analyses of prepared diets and resultant eggs are ongoing.

Study Number: LM-04-09

Title: Contaminant Loads in Broodstock Fish in the Region 5 National Fish Hatchery System

Principal Investigators: Michael J. Millard and John W. Fletcher (NEFC)

Cooperators: Timothy Kubiak, Pleasantville, NJ Field Office, Steve Mierzykowski, Old Town, ME Field Office,

Background & Justification

A Jan. 4, 2004 report in *Science*, and related media releases, raised consumer safety concerns regarding farm-raised salmon and fish consumption in general. The report documented elevated contaminant levels, including PCBs and dioxins in farm-raised salmon, and cited the likely source for the contaminants as the fish oil used in the commercial feed manufacturing process, with subsequent bioaccumulation by the salmon. While a plan for monitoring contaminant loads in USFWS Region 5 broodstock and feed sources is being developed, there was concern over levels in existing surplus broodstock. Since these broodstock are transferred to the States for recreational fishing with possible public consumption, Region 5 fisheries administrators decided to evaluate the fish for levels of PCBs, dioxins, and/or heavy metals and compare results with federal consumption advisories.

Objectives

The contaminant loads in 138 adult fish from the R5 National Fish Hatchery system which are or could be destined for release into the wild were evaluated in 2004 for contaminant loads of organochlorines and mercury. Species and numbers evaluated were: 90 Atlantic salmon, 24 lake trout, and 24 rainbow trout.

Methods

Fish were sampled in late February and early March, 2004, by staff from NEFC. Fish tissue samples were collected using standard contaminant sampling protocols; these protocols were consistent with EPA sampling standards (*Guidance for Assessing Chemical Contaminant Data for Use In Fish Advisories. Volume 1: Fish Sampling and Analysis - Third Edition, National Guidance, EPA. S*amples were processed through the Patuxent Analytical Control Facility using standard QA/QC procedures, as specified in their laboratory contract. Geometric means were calculated for species groups and categorized into EPA consumption advisories.

Results

PCBs *Atlantic salmon*.- Geometric mean level in all salmon samples was 0.0335 ppm. Highest levels found in the salmon was 0.0896 ppm recorded in the 4-year-old sea-run fish in Nashua NFH . The domestic broodstock in Nashua NFH ranked as the 2nd highest group of fish with respect to total PCBs. Domestic broodstock from White River NFH ranged between 0.0225 and 0.0364 ppm while younger fish from Green Lake NFH showed the lowest levels. No samples triggered “do not eat” EPA advisory (>0.094ppm wet wt for cancer health endpoints). With respect to the EPA noncancer endpoints, all salmon were in the 2, 3, or 4 meals/month categories. *Lake trout*. - Levels in lake trout from Allegheny NFH ranged between 0.0431 and 0.0832ppm with older fish (age 8) ranking highest and younger (age 3) ranking lowest. Three samples triggered an EPA advisory of 0.5 meal/month and the remaining sample fell within the 1 meal/month advisory category. *Rainbow trout*.- Levels in trout from White Sulphur Springs NFH ranged between 0.0456 and 0.0727ppm with the geometric mean within the 0.5 meal/month EPA advisory. **Dioxins**. *Atlantic salmon*.- Geometric mean concentration in all samples was 0.5330ppt TEQs falling within the 1 meal/month EPA advisory. Sea-run adults from Nashua fell within the “do not eat” category. *Lake trout*.- 3 lake trout samples triggered an EPA cancer risk advisory of 0.5 meal/month and the remaining sample was in the 1meal/month advisory category. *Rainbow trout*.- The geometric mean concentration for the trout samples was 0.7759, falling within the 0.5 meal/month EPA advisory.

Summary - In terms of triggering EPA consumption advisories, dioxin levels from individual samples in Region 5 NFH broodstock were generally as restrictive, or more so, than were the total PCB levels.

Study Number: LM-04-10

Title: Evaluation of genetic diversity and relatedness for Atlantic sturgeon (*Acipenser oxyrinchus*) captively held by Maryland DNR

Principal Investigator: Meredith Bartron, Northeast Fishery Center (NEFC)
Co-Invest/Cooperators: Shannon Julian (NEFC); Brian Richardson -MD Dept. of Natural Resources **(**MDDNR)

Background and Justification

Hatchery supplementation has often been used as a tool in fisheries management to restore declining populations, supplement existing populations, or introduce new species. An important component of hatchery supplementation is broodstock management with goals of restoration or recovery of declining populations while minimizing inbreeding potential and maintaining genetic diversity. To attain these goals, broodstock techniques focus on the number of adults used for spawning and the mating strategy used for propagation. MDDNR currently maintains a captive broodstock of Atlantic sturgeon. Offspring resulting from captive broodstock propagation would be incorporated into a stocking program for the greater Chesapeake Bay area. Adult and juvenile sturgeon were obtained by MDDNR from two sources: wild-caught juveniles from the Chesapeake Bay, and from captive propagation of Hudson River adults.

Study Objectives

In 2004, we used microsatellite markers to estimate measures of genetic diversity for 197 individuals from both the wild caught and captive-bred groups of sturgeon by MDDNR for use as broodstock. Information including genetic variation (heterozygosity), relatedness (proportion of shared alleles), and genetic distance were used to quantify each group.

Materials and Methods

A total of 197 tissue samples were taken from wild and captive Atlantic sturgeon and genotyped using 15 microsatellite loci. Allele frequencies, observed heterozygosity, an # of alleles/locus were calculated using GENEPOP and GDA software. Pairwise comparison of F_{ST} values (differences in allele frequencies) between captive-bred group and wild caught fish were calculated using FSTAT v.2.9.3.1 software. Pairwise relatedness values (R_{xy}) for fish in the captive-bred group and the wild caught group were calculated using Relatedness software. Pair-wise genetic distances between individuals were calculated using the transformed proportion of shared alleles (-ln (PSA)) algorithm in Microsat ver.1.5d software. An unrooted tree was fit to the genetic distance matrix using the NEIGHBOR routine from the PHYLIP computer program and visualized using the TreeView computer program.

Results

- In total, 31.5 % of the fish genotyped were trisomic, and of those, 91.9% were part of the captive-bred group. Only 8.06% of the trisomic individuals were of wild caught origin.
- Statistical analysis of the genetic results was possible for 135 individuals, 85 from the captively bred group, and 50 from the wild caught group.
- The wild-caught population had an average of 14 alleles per locus for 15 loci, observed heterozygosity of 0.794 (H_o), and fixation index (f) of 0.063.
- Within the captively bred population, there was an average of 8.73 alleles per locus for 15 loci, observed heterozygosity of 0.787, and fixation index of –0.043. The fixation index is the reduction in heterozygosity expected with random mating within a group.
- Pairwise comparison of population differentiation based on differences in allele frequency between the wild-caught and captively bred groups (F_{ST}=0.035) was statistically significant (P=0.05). Mean pairwise relatedness was greater within the captive bred group (R_{xy}=0.107, CI=0.033), than within the wild caught group (R_{xy}=-0.016, 0.011 C).
- Largest genetic distances were found between wild animals and the smallest were between captive bred individuals. Distances between wild individuals ranged from 0.388 to 10.000 and averaged 1.603 whereas distances among captive bred animals ranged from 0.113 to 2.639 and averaged 1.075.

Study Number: LM-04-11

Title: Genetic structure of the horseshoe crab (*Limulus polyphemus*) populations in Delaware Bay

Principal Investigator: Meredith Bartron, Northeast Fishery Center (NEFC)
Co-Invest/Cooperators: Mike Millard (NEFC); Dave Smith (USGS)

Background and Justification

Horseshoe crabs (*Limulus polyphemus*) abundance in Delaware Bay has declined over recent years. Horseshoe crabs represent an integral component to the Delaware Bay ecosystem. For example, horseshoe crab eggs provide a major food source for migrating shorebirds. However, declining numbers of horseshoe crabs have reduced the amount of eggs available to shorebirds. Tagging studies have been initiated to examine adult horseshoe crab movement with Delaware Bay and fidelity of adult crabs to spawning beaches. Observations of movement are helpful to understand habitat utilization and spawning site fidelity, but observational studies are unable to provide information regarding successful reproduction and population structure of crabs in the bay. Additionally, because harvest of horseshoe crabs in Delaware Bay is regulated by multiple agencies, it is important to know if management efforts are focused on a single panmictic population (if crabs randomly choose spawning sites), or if multiple populations of horseshoe crabs exist in Delaware Bay. Understanding the population genetic structure of horseshoe crabs will aid in management of the crabs, provide critical information regarding life history traits, and aid in restoration efforts.

Study Objectives

Our goal for this project is to determine the spatial genetic relationships among horseshoe crab spawning beaches in Delaware Bay. Multiple sampling times throughout the spawning season will help determine the population structure of spawning beaches, and help determine if distinct populations exist temporally throughout the spawning period. This study represents the first year of a multi-year project to determine the temporal and spatial relationships of horseshoe crabs in Delaware Bay.

Materials and Methods

Adult horseshoe crabs were non-lethally sampled from spawning beaches located around Delaware Bay. Approximately 60 individuals (equal sex ratios of male and female crabs) will be sampled from three beaches in Delaware: Kittshummock, Big Stone, and Fowler, and from three beaches in New Jersey: Fortescue, Reeds, and Highs beaches. Sampling occurred during the peak spawning events in the spring of 2004: May 3-6, May 17-21, and June 1-4. Small tissue samples were clipped from the pinchers of the adult crabs, and stored in 95% Ethanol. Microsatellite markers are being used to quantify allele frequencies and estimate genetic variation among horseshoe crab spawning beaches.

Results

This study is ongoing and is estimated to be completed in the summer of 2005.

Study Number: LM-04-12

Title: Genetic characterization of an isolated population of Northern Plymouth Red-bellied Cooter (*Pseudemys rubriventris*) in Massachusetts

Principal Investigator: Meredith Bartron, Northeast Fishery Center (NEFC)
Co-Invest/Cooperators: Michael Amaral (USFWS-New England Field Office); Shannon Julian (NEFC)

Background and Justification

The Northern Red-bellied cooter is currently listed as endangered in Massachusetts. There is some discussion regarding whether or not the Massachusetts population is a subspecies, or represents a single isolated population. Because the Massachusetts population is isolated and its status is supplemented by a head-start hatching program, there are additional concerns about the genetic diversity and long-term viability of the population, particularly through high levels of inbreeding. Information about the genetic diversity observed in the Massachusetts population will be used by agencies to revise management and conservation strategies as needed.

Study Objectives

The goal of this project is to characterize the genetic diversity observed in an isolated turtle population in Massachusetts relative to populations observed in other parts of its range. Due to the isolated nature of the population, there is an increased potential for inbreeding to occur in the population, which could result in decreased fitness of individuals in the population, increasing the potential for localized extinction.

Materials and Methods

Samples will be obtained from juvenile turtles in the head-start program run by Massachusetts Department of Wildlife to characterize the isolated population. In addition, samples are being obtained from populations in Maryland, Pennsylvania, Virginia, New Jersey, and North Carolina for comparison to the Massachusetts population. Multiple microsatellite markers will be used to obtain genotypes used to characterize individuals from each population, and data will be analyzed to determine the partitioning of genetic variation observed within and between populations.

Results

This study is ongoing and is estimated to be completed in the fall of 2005.

Study Number: LM-04-13

Title: Genetic characterization of two northern riffleshell (*Epioblasma torulosa rangiana*) populations in the Allegheny River

Principal Investigator: Meredith Bartron, USFWS-Northeast Fishery Center (NEFC)
Co-Invest/Cooperators: Catherine Gatenby (USFWS-White Sulphur Springs)

Background and Justification

The northern riffleshell (*Epioblasma torulosa rangiana*) is currently listed as an endangered species. Two isolated populations are thought to be the only populations of northern riffleshell remaining in the Allegheny River, a tributary to the Ohio River. The two populations in the Allegheny River are currently threatened by road construction projects. To prevent potential harm to the population due to increased sedimentation due to bridge construction, both groups of mussels will be temporarily removed from the area and housed at the USFWS White Sulphur Springs (WSSNFH) mussel culture facility.

Study Objectives

The use of refugia and captive propagation for mussel culture presents unique opportunities to obtain genetic material from mussels. We propose to characterize estimates of genetic variation in each of the populations to identify relative isolation of the populations, estimate inbreeding coefficients, and determine genetic viability of each population. Additionally, captive breeding protocols will be developed to ensure long-term maintenance of genetic diversity.

Materials and Methods

Tissue samples will be obtained non-lethally from the captive northern riffleshells held at WSSNFH. Variable microsatellite markers will be used to characterize genetic diversity of the two captive populations. Genetic information for each population will be used to estimate gene flow between populations and levels of genetic diversity within and between the two populations.

Results

This study is ongoing and is estimated to be completed in the spring of 2006

Study Number: LM-04-14

Title: Assessment of Watershed Scale Habitat Features on the Survival of Juvenile Atlantic Salmon

Principal Investigator: Meredith Bartron and John Sweka, USFWS-Northeast Fishery Center (NEFC),
Co-Invest/Cooperators: Joan Trial (ASC)

Background and Justification

The ultimate measure of the success of recovery efforts for Atlantic salmon is the number of returning adult fish to the river. The number of returning adults is positively correlated to the number of outmigrating smolts produced. The abundance of outmigrating smolts is a combination of the number of parr surviving to the smolt stage as well as the survival of smolts during emigration from the various portions of the watershed. Smolts from various portions of the watershed will experience differing factors limiting survival. Thus, even if survival to the parr stage is high for a particular area of the river, this area may not contribute a significant portion of the total smolt population if high mortality occurs during migration. In order for recovery efforts to be successful there is a need to identify areas of the watershed which contribute most to the outmigrating smolt population. Identification of such areas will allow managers to refine fry stocking practices to increase the number of outmigrating smolts per the number of fry stocked and will help guide future salmon habitat enhancement and restoration efforts.

Study Objectives

(1) Determine quantitative relationships between inter-stage survival of juvenile Atlantic salmon and macrohabitat variables such as watershed area, temp., pH/Alkalinity, stream gradient, and abundance of non-salmon / predatory species. **(2)** Examine spatial patterns of juvenile salmon growth in terms of absolute growth and as a % maximum potential growth based on stream temperatures. **(3)** Use genetically marked fry to identify the rearing locations of outmigrating smolts and assess relative survival from various stocking sites. **(4)** Make recommendations to optimize smolt production from fry stocking.

Materials and Methods

This study will be conducted over 3 yrs. and will assess juvenile Atlantic salmon inter-stage survival from fry stocking through outmigrating smolts as it relates to habitat within the entire Sheepscot R. watershed. The watershed will be broken into several major reaches according to previously mapped rearing habitat and other natural features. The 1st portion of the study is to observe spawning activities to ensure tracking of parentage for identification of juveniles. Sheepscot R. broodstock at Craig Brook NFH will be genotyped using highly polymorphic microsatellite DNA markers. Combined knowledge of the parental genotypes and hatchery matings will allow genetic parentage to be determined on the resulting juveniles at all life stages. Using genetic parentage analysis as a “mark”, we will be able to evaluate survival from each stocking location. Within each river reach, a single genetic group (known families) will be stocked in the spring 2005 at an average density of 100/100m^2. A family will be within only one genetic group and stocked in only one river reach. Age-1 parr survival will be assessed during fall, 2006 at a minimum of 3 sites within each reach. The size of each site will vary according to habitat features and will approximate 1 habitat unit (1 unit=100m^2). Multiple electrofishing passes will be made to collect parr for measurement. Non-lethal genetic samples will be taken to determine the degree of immigration of fish stocked in other river reaches. The age-1 population at a site will be estimated using the generalized removal estimator in the program CAPTURE. Parr density will be equal to the population estimate divided by the area of the site sampled. The 2nd sampling stage of the study will assess survival to the smolt stage using a rotary screw trap operated by NOAA fisheries near Head Tide Dam on the mainstem Sheepscot in the spring 2007 since >90% of salmon in the Sheepscot smoltify at age-2. Smolt trapping will be conducted Apr.–June corresponding to the historical timing of outmigrating smolts. Upon collection, smolts will be non-lethally weighed and measured and a fin clip taken for genetic analysis.

Results

This study began with the spawning of adults in the fall of 2004, and will continue until the winter of 2007.

OTHER BIOLOGICAL AND RELATED INVESTIGATIONS PERFORMED:

LM04A Fish Health Inspection/Monitoring/Diagnostic Services.- The Lamar Fish Health Center processed 268 laboratory cases in fiscal year 2004. Region 5 has a very extensive fish health monitoring program to enhance the fish health inspections, allowing continual surveillance of the health status of the stocks, some of which have been identified as very limited distinct population segments (DPS) which the Service has listed under the Endangered Species Act (ESA). The Fish Health Center had 32 lot by lot inspection cases. These are conducted to allow interstate transfer as well as release of fish. Ten federal (or cooperating with a federal program) facilities received these on-site statistically based investigations and another 10 were conducted, as outlined in the Service Fish Health Policy, as virology lab services only for non-Service entities. These examinations are essential to prevent the spread of fish diseases through fish and/or egg transfers and are necessary to enable facilities to comply with regulations on transporting and releasing fish. In addition to the 172 monitoring cases involving examination of fish, 2 Service facilities provided 28 water monitoring cases, where water from rearing units was examined by the water filtration method, a very effective proactive protocol for diagnosing furunculosis before an epizootic occurs. In fiscal year 2004 thirteen laboratory diagnostic examinations were conducted for 6 facility/agencies, where moribund fish were examined and tested to determine the cause(s) of mortalities and other problems and recommendations for resolution were provided. Contact: John Coll

LM04B Sonic tagging of hatchery-reared and wild juvenile Atlantic sturgeon to track fish movements in the Hudson River.- NEFC's Fish technology section assisted the state of New York Department of Environmental Conservation (DEC) to perform a study which allowed tracking of individual Atlantic sturgeon in the Hudson River. NEFC surgically implanted sonic transmitters into 24 hatchery-reared sub-adult sturgeon and transported them to New York for release at Hyde Park on the Hudson River. NEFC also implanted the transmitters into 9 wild-captured juveniles for a tracking study by DEC. Preliminary results from DEC showed that 5 months after release, 20 out of 24 hatchery fish had been located from Yonkers to Poughkeepsie. During the summer months hatchery fish were distributed from Hastings on Hudson to Kingston and all but two of the regularly observed fish had moved downriver. Seven fish moved fish < 1km and 13 fish have moved >10km. Also after about 5 months, 7 out of 9 wild-tagged fish were found. Wild fish were distributed from Dobbs Ferry to Newburgh. Most wild fish had moved into the Hudson Highlands or southern Newburgh Bay during the summer months. Four out of the nine tagged fish have moved back into Haverstraw Bay where they were first tagged. Contact: Jerre Mohler

LM04C Biopsy of captive Atlantic sturgeon to determine reproductive maturity.- Nine captive wild Atlantic sturgeon from 3 sources were biopsied for gender determination and degree of sexual maturity in June, 2004. Two fish were determined to be females, one of which had oocytes in a pre-vitellogenic stage indicating that egg maturity may be completed in 2005 or 2006. Four fish were determined to be males and 3 fish remained undetermined due to excessive bleeding and heavy ossification in biopsy areas precluding surgery. Contact: Jerre Mohler

LM04D Digital photography of calcein marks on endangered desert fish.- Preserved samples of endangered desert fish were received from USFWS - Dexter Fish Technology Center in New Mexico. Species were: Bonytail chub, Rio Grande silvery minnow, woundfin minnow, and Colorado pikeminnow. Fish had been marked with calcein at Dexter and microphotos of the calcein marks were needed. NEFC provided the technical assistance by photographing the samples on our epi-fluorescent microscope outfitted with a 35 mm digital camera. Contact: Jerre Mohler

LM04E Evaluation of calcein-marks on 5-yr-old Atlantic salmon.- Five-yr-old Atlantic salmon which had been calcein-marked in the parr (1-yr-old) life stage and maintained indoors were examined for visible calcein marks with a SE-MARK™ hand-held detector and epi-fluorescence microscope. Ten of the 11 fish examined with the detector had easily-detectable fin marks and 6 had detectable scale marks. Using the microscope, 10 of the 11 fish had easily-detected scale and fin marks. Contact: Jerre Mohler

OTHER BIOLOGICAL AND RELATED INVESTIGATIONS PERFORMED (continued):

LM04F Sturgeon gill-netting and sampling on the Delaware River.- Two weeks of assistance was given to the Delaware River Coordinator to provide sturgeon gill netting expertise and equipment preparation. Even though an occasional mature Atlantic sturgeon is found dead or injured in the lower Delaware River, no locations have been identified where broodstock can be reliably captured. Therefore, searching for broodstock by the USFWS and Delaware State University will be conducted subsequent to preliminary assistance for a number of years. Contact: Jerre Mohler or Patrick Farrell

LM04G Partnership with the Pennsylvania Fish and Boat Commission for pond culture of striped bass. - Culture activities under a Memorandum of Understanding continued between the PA Fish and Boat Commission and the Northeast Fishery Center to provide use of five NEFC ponds for culture of striped bass juveniles for the Commonwealth. Contact: John Fletcher

LM04H Statistical/Study design consultations – The population ecology section's biometrician performed 7 statistical consultations for intra- and interagency partners. These consultations included study design recommendations, estimation of sample size requirements, and review and comment on analytical methodologies. Also, two manuscript reviews were conducted for peer reviewed journals. Contact: John Sweka

LM04I Development of a non-lethal measure of estimating Atlantic salmon proximate composition for use in fish condition assessment - Faculty from West Virginia University are using bioelectrical impedance analysis (BIA) to create a statistical model which can then be used to estimate body composition of Atlantic salmon by non-lethal means. Separate groups of Atlantic salmon were fasted or fed at elevated rations at the NEFC to create variation in body condition. When the fish showed visual differences in condition, they were anesthetized and analyzed with BIA techniques. Approximately 20 juvenile and adult fish were analyzed from each fasted/fed group. The fish were then sacrificed and frozen for later laboratory determination of proximate body composition. Once the laboratory analysis is complete, then a model will be developed which can be used proximate composition determination without sacrificing the fish for such analysis. Development of this technology will be beneficial to both hatchery and field investigations concerning condition of Atlantic salmon. Contact: John Sweka

LM04J Northeast Fishery Center web page development - Jeremy Craven, Kelly Pennypacker, Ryan Diehl, George Rusczykm and Robert Harley, five computer science majors from Lock Haven University, volunteered a combined 238 hours. Working jointly with Paula F. Bell, Associate Professor of Computer Information Science, and NEFC Volunteer Coordinator, Wade Jodun, The group created a draft Homepage for the Center which was then modified by the Lower Great Lakes Fish and Wildlife Office into a final product. The web page **(http://northeast.fws.gov/fisherycenter/)** became operational May 26, 2004. Summaries of the Center's current biological activities and the staff's scientific publications are now available on the site. Contact: Wade Jodun

LM04K Development of water/air supply systems for freshwater mussel culture – In July and August, 2004, NEFC biotechnician Pat Farrell, designed and installed complete pressurized water systems and low pressure, high volume air distribution systems for the 2 new mussel culture buildings at White Sulphur Springs National Fish Hatchery, WV In addition, a joint project with Dave Orcutt, professor emeritus from VA Tech, was completed for the design and construction of 4 roll-around algae culturing systems which included 3-tiered racks for the mussel culture tanks, installation of the tanks, water distribution, and drain lines. Other equipment installed included a distiller & holding tank with a feed line to the lab, a UV sterilizer with holding tank, and special filters on the air system in the green house. Contact: Patrick Farrell

OTHER BIOLOGICAL AND RELATED INVESTIGATIONS PERFORMED (continued):

LMO4L Participation in the National Wild Fish Health Survey.- This project, launched in 1997, continues to involve all nine Service Fish Health Centers nationwide, incorporating standardized diagnostic techniques and data management methods to ensure comparability. In fiscal year 2004, the Fish Health Center initiated 24 cases for the Survey, in which 426 fish (8 different species) from a total of 23 sites were examined and efforts continued to enter completed cases into the NWFHS database. This database which is capable of single and double queries based on either fish species or fish pathogens, is now accessible via the internet on the Service website. The National Wild Fish Health Survey is partnership driven and fiscal year 2004 enabled the Lamar Fish Health Unit to conduct cooperative work with West Virginia Department of Natural Resources and Vermont Fish and Wildlife Department on assessing the prevalence of largemouth bass virus in bass residents of several bodies of water and with several state and federal natural resource agencies (CT,ME,MA,NY,VT) in developing brood stocks from feral populations. Outreach activities to increase awareness of the National Wild Fish Health Survey and involve other Service and partnering programs continue. Contact: John Coll

LM04M Incidence and Prevalence of Infectious Salmon Anemia virus (ISAv) in Sea Run Atlantic Salmon held at Service NFHs as Broodstock. –The non-lethal ISAv surveillance protocol for screening sea-run Penobscot River Atlantic salmon as they are captured and brought to U.S. Fish and Wildlife National Fish Hatcheries to determine incidence was expanded in fiscal year 2004. At Craig Brook NFH in Maine, a sub-sample (60) of fish were sampled non-lethally (blood)and tested by reverse transcriptase-polymerase chain Reaction (RT- PCR) and cell culture on SHK-1 and ASK cells. All fish tested negative by both PCR and cell culture techniques. As a tool for managing this virus at the facility, the entire population (n=602) was similarly screened, following cohabitation and prior to spawning. This year Connecticut River sea-run Atlantic salmon broodstock held at Cronin National Salmon Station in Massachusetts (n=61) and Merrimack River sea-run salmon held at Nashua NFH in New Hampshire (n= 120) were also non-lethally screened for ISAv. All tested negative for the virus and no isolation / quarantine of eggs was deemed necessary. Contact: John Coll

LM04N U.S. Fish and Wildlife Service Fish Health Procedures Handbook. -In cooperation with all nine USFWS Fish Health Centers, and in collaboration with the American Fisheries Society - Fish Health Section, a procedural manual for Fish Health Inspection Protocols has been further developed. A representative of the Lamar FHC chaired subcommittees to gather and edit procedures for the Sampling, Bacteriology and Quality Assurance Chapters of this Manual. The intent of the Handbook is to establish a nationally consistent set of protocols for use by all fish health inspectors and diagnostic laboratories when performing fish health inspections of fish culture and aquaculture facilities. The document will be provided to the Fish Health Task Force of the Congressional Joint Subcommittee on Aquaculture (JSA) for their review and adoption as a national standard for Fish Health Inspections. The document has also become a chapter in the updated version of the AFS/FHS “Bluebook”. It is available for viewing on the Internet at http://fisheries.fws.gov/FWSFH/NFHSmain.htm Contact: John Coll

LM04O Quality Assurance/Quality Control for Infectious Salmon Anemia virus (ISAv) Samples and Diagnostic Techniques. - The Lamar Fish Health Center participated in a multi-laboratory ring testing project to determine the consistency of laboratory techniques involved with detection of infectious salmon anemia virus from fish tissues. Cooperators on the project were the National Marine Fisheries Service and the Atlantic Veterinary College, University of Prince Edward Island, PEI – Canada. It involved receiving hundreds of blind samples for analysis by RT-PCR for ISAV. Results were evaluated for inter and intra-lab consistency, and evaluation of inconsistency in procedures. The study resulted in very positive quality control performance by the Lamar Fish Health Center, as compared to other laboratories participating. The study will result in a publication in the Journal of Fish Diseases, and is entitled “Estimation of the repeatability and reproducibility of three diagnostic tests for infectious salmon anemia virus” and is currently in review. Contact: Patricia Barbash

OTHER BIOLOGICAL AND RELATED INVESTIGATIONS PERFORMED (continued):
LM04P Fish Health Extension Services -The Lamar Fish Health Center continues to provide extension services to all federal, state, tribal and private inquiries in the area of fish health. Services provided include technical consultations, provision of supplies for fish necropsies, treatment recommendations and calculations, antibiotic injections, vaccinations, development of biosecurity plans, review of international and interstate fish importations, and furnishing procedural protocols. Contact: John Coll

LM04Q U.S. Fish and Wildlife Service Title 50 Revision Committee - The Lamar Fish Health Center actively continued in the Service-wide effort to revise the Service's national importation regulations for fish, egg, and gamete importation, 50CFR16.13. This process, now in its infancy consists of development of a position paper and identification of appropriate partnering agencies. Work will be cooperative with the newly developed National Aquatic Animal Task Force (NAAHTF) established through the Joint Sub-committee on Aquaculture (JSA). Contact: John Coll

LM04R Vaccination of pre-release Connecticut River smolts using multi-valent vaccine – The Lamar Fish Health Center, in cooperation with partners involved with the Connecticut River Atlantic Salmon Committee, coordinated and administered injectable vaccine to 100,000 Atlantic salmon smolts the Pittsford NFH. This is the third year of administering this vaccine. The vaccine will protect the fish during their stay at the hatchery, as well as up to a year after they are released into the river from the bacterial pathogens that cause furunculosis and vibriosis. Evaluation of the efficacy of the vaccine in improving salmon survival in the wild will be based on adult sea run returns beginning in the 2005 migration. Contact: Patricia Barbash

LM04S Craig Brook NFH Atlantic salmon 2002 broodstock evaluation.- The Conservation Genetics Lab of the USFWS completed genotyping of the 2002 Atlantic salmon broodstock being held at Craig Brook NFH. Parr were genotyped from the East Machias, Machias, Pleasant, Dennys, Sheepscot, and Narraguagus, and samples from adult salmon were obtained from the Penobscot River. Genotype data is used to monitor measures of genetic diversity for each year class of broodstock, and track recovery of family groups as parr from fry stocking. Additionally, genotypes were obtained from the group of parr from the Pleasant River that were kept in the hatchery rather than stocked out. Genotypes from the fully captive Pleasant group were compared to those from the fry that were stocked out into the Pleasant River and recaptured as parr to evaluate recovery of families. Contact: Meredith Bartron

LM04T Development of a computer program to identify genetically optimal matings for broodstock management.- Inappropriate hatchery mating designs can reduce effective population size, increase the likelihood of inbreeding and create population bottlenecks. New approaches, incorporating genetic data on potential breeders reduce chances of inbreeding, and have already been incorporated successfully in the Connecticut River and Maine Atlantic salmon restoration efforts. As part of the optimal mating software, we propose to develop an improved mating design optimization procedure. The improved design algorithm will incorporate factorial mating design and will use optimization procedures to determine the best set of mating pairs across all fish available for mating on a particular day. This will improve the efficiency of the program and facilitate it's use in the hatchery environment. The new software will match already genotyped candidate parents for matingwhile (1) maximizing genetic distance (2) minimizing relatedness (based on relatedness measures and pedigree data) (3) following a user-defined mating strategy (4) allowing input flexibility for adults ripe that day (5) calculating both single-pair and factorial optimized sets of matings (6) tracking of "lifetime contribution" of individuals (7) tracking precocious parr and factoring in this information after some threshold contribution of parr has been surpassed (8) generating summary information on actual pairwise matings. The computer program was developed in 2004 through a SSP project with Dr. Ben Letcher of USGS-Conte Lab and his graduate student, Jason Coombs, and tested on a trial basis for the fall 2004 spawn. This project will continue into FY 2005, and then the program will be available for implementation in the fall 2005 spawn. Contact: Meredith Bartron

OTHER BIOLOGICAL AND RELATED INVESTIGATIONS PERFORMED (continued):

LM04U Craig Brook NFH Atlantic salmon 2003 broodstock evaluation.- The Conservation Genetics Lab of the USFWS has begun genotyping of the 2003 Atlantic salmon broodstock being held at Craig Brook NFH. Parr that were captured in the fall of 2003 are being genotyped from the East Machias, Machias, Pleasant, Dennys, Sheepscot, and Narraguagus Rivers. Samples from adults from the Penobscot River that were spawned in 2004 will also be genetically characterized. Genotype data will be used to monitor measures of genetic diversity for each year class of broodstock, and track recovery of family groups as parr from fry stocking. Contact: Meredith Bartron

LM04V Genetic characterization and marking of Merrimack River Atlantic salmon.- Atlantic salmon were extirpated from the Merrimack River in the mid-1800's when dams constructed for hydropower blocked salmon from returning to upstream spawning habitat. The objective of this study is to determine the effectiveness of the hatchery stocking and breeding program on the Merrimack River. This project characterizes sea run broodstock, kelt broodstock, and adults returning to the river. Parentage analysis will be used to identify parents of returning adults, which will allow determination of parental source (sea-run or kelt), and stocking location of the family group to evaluate stocking habitat on the survival to adult stage. Samples have been obtained from the broodstocks, and genotyping will continue into the summer 2005. The project is conducted in conjunction with Nashua National Fish Hatchery. Contact: Meredith Bartron

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